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Free-field power density has long been used as an index of energy dosing in studies of biological effects of microwave radiation. However, this method of quantifying dose can lead to considerable error if it is used as an index of the rate of energy actually being absorbed by a specimen, because the relative absorption cross sections of different specimens may vary greatly. The integral of absorbed energy is a more meaningful measure of exposure; it can be accomplished by using such sophisticated equipment as twin-well calorimeters, or by using the cruder system of saline-filled phantoms. This paper describes a calorimetric system for the measurement of absorbed energy in laboratory animals and in in vitro samples that can be assembled from such common laboratory equipment as Dewar flasks, magnetic stirrers, foamed polystyrene, and an accurate temperature measuring device. Measurement of absorbed energy by this system for a standard tissue-culture flask are compared to values obtained by electrical measurements, by direct temperature measurements, and by heating and cooling curves. Also, application of the system to measurements of dose in experimental animals is demonstrated.

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Measurement of microwave radiation absorbed by biological systems, 2, Analysis by Dewar-flask calorimetry

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Free-field power density has long been used as an index of energy dosing in studies of biological effects of microwave radiation. However, this method of quantifying dose can lead to considerable error if it is used as an index of the rate of energy actually being absorbed by a specimen, because the relative absorption cross sections of different specimens may vary greatly. The integral of absorbed energy is a more meaningful measure of exposure; it can be accomplished by using such sophisticated equipment as twin-well calorimeters, or by using the cruder system of saline-filled phantoms. This paper describes a calorimetric system for the measurement of absorbed energy in laboratory animals and in *in vitro* samples that can be assembled from such common laboratory equipment as Dewar flasks, magnetic stirrers, foamed polystyrene, and an accurate temperature measuring device. Measurements of absorbed energy by this system for a standard tissue-culture flask are compared to values obtained by electrical measurements, by direct temperature measurements, and by heating and cooling curves. Also, application of the system to measurements of dose in experimental animals is demonstrated.

1. INTRODUCTION

Energy absorption is a fundamental measure that should be included in all reports of biological effects of radiation. *Johnson* [1975] has described the rationale for including this information, while *Justesen* [1975], *Süsskind* [1975] and *Guy* [1975] have discussed terminology. *Johnson and Shore* [1975] instructed authors to include information on absorbed energy in papers submitted for inclusion in the Proceedings of the 1975 Annual Meeting of URSI. Thus, there is general recognition that a rigorous description of microwave exposures should include values for the averaged quantity of energy absorbed by a specimen in addition to the more common descriptions of the incident microwave field and of the specimen's configuration.

The average of the energy absorbed by a specimen can be calculated most easily from the rise or fall of temperature (ΔT) that is associated with exposure, or with a cooling curve during a period after exposure to radiation. Although these methods measure only the thermalized portion of absorbed energy and ignore other modes of storage or dissipation, in general, thermalized energy is a useful index of absorbed energy. *Allis et al.* [1977] have described a method for calculating absorbed energy by analyzing the rate of temperature change as indicted by heating and cooling curves. This heating/cooling method has wide applicability, but may not be useful if the temperature probe interacts with the microwave field or if the specimen either restricts placement of the probe or limits the rate of internal thermal mixing (as might be the case for intact animals). These difficulties can be overcome by using a calorimetric method that requires only the averages of the initial and of the final temperatures of the specimen.

Our paper describes a calorimetric method for measuring absorbed energy by a Dewar flask. The method is simple, inexpensive, and can be used with many types of samples. The results of measurements of absorbed energy in a simple *in vitro* sample are compared with those obtained by several other methods: the heating/cooling method, direct temperature measurement of the sample, and electrical measurement of power deposition in a transmission-line exposure system. The ability of the calorimeter to measure absorbed energy in intact animals is also demonstrated.

2. MATERIALS

2.1. *Measuring equipment.*

The main component of the calorimeter is a vacuum (Dewar) flask containing a measured amount of water. The flask is sealed wth a cork stopper to minimize heat exchange with the environment and the water is thoroughly mixed by a magnetic stirrer to minimize the time for the contents to reach thermal equilibrium. The temperature of the water is measured with a thermistor device (Yellow Springs Instrument Company, Tele-Thermometer Model TUC46, with Probe Type 421) connected to a chart recorder; however, any device can be used that will indicate temperature with an accuracy of ± 0.01 °C.

2.2. *Microwave exposure systems.*

Two types of microwave facilities were used to expose specimens. Exposures to radiation at 2.45 GHz were conducted in the far field of a horn in an anechoic chamber with a regulated source of microwave energy. A dipole probe was used to determine free-field power density, as described by *Blackman et al.* [1975]. Exposures at 1.0 GHz were conducted in a rectangular, coaxial transmission-line exposure system that was designed and constructed in our laboratory. This system contains a TEM-mode propagating wave; bidirectional couplers (NARDA 3020A) and thermoelectric power meters (HP 435A) were used to monitor forward, reflected, and transmitted powers, from which rates of energy absorption by a sample load can be approximated.

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2.3. *Samples.*

The samples were either: T-flasks (Falcon), filled with 40 ml of 0.15 M KCl to approximate the conductivity of most physiological solutions or dead bodies of Chinese hamsters that were sealed in air-evacuated plastic wrap (Saran) to contain excrement. One T-flask was used to corroborate the calorimetric method and was fitted with a YSI #520 probe that projected into the middle of the flask from its bottom, where it was sealed with an adhesive of silicone rubber. All T-flasks were at ambient temperatures (23° to 26 °C) when exposed. All samples were placed in a three-piece foamed polystyrene container during far-field exposures at 2.45 GHz to minimize heat loss. Two and one-half to 5-cm (one- to two-inch) thick foamed polystyrene sheets were used without any apparent change in the results. No adjustment was made for the presence of Styrofoam because when dry it is virtually transparent to microwave radiation. The geometry of the exposure situation was carefully reproduced for each exposure.

3. METHODS AND PROCEDURES

A single mathematical relation is used to calculate the thermal energy deposited in a sample by microwave radiation. This relation, which uses the initial and final mean temperatures of the total system, is essentially the same as that used in introductory physics courses to calculate specific heat. The averaged quantity of energy absorbed by a sample, E, is computed from the averaged temperature of the sample immediately before (T_b) and after (T_e) radiation, the composite specific heat, C, and the mass, M, of the sample. The relationship is:

$$E = M \times C \times (T_e - T_b) \qquad (1)$$

The averaged rate of energy absorption can be calculated from E by dividing it by the duration of exposure, so long as the ΔT is linearly proportional to the exposure time. The linear relation depends on factors such as the surface area, emmissivity and absorptivity of the specimen and the temperature difference between the heated specimen and its environment. Thus, each type of specimen should be examined to determine the range of exposure times that will provide a linear response. Possible artifacts caused by heat loss or changes in absorption as a function temperature do not contribute significantly to the measurement if the same absorption rate is calculated from exposures for different periods of time. The temperature before exposure is determined easily because the sample can be placed in a constant temperature environment until a uniform temperature is reached. However, the averaged temperature after exposure cannot be obtained as easily, especially for samples with complex compositions, since temperatures throughout the sample may differ because of the complex pattern of deposition of microwave energy.

3.1. *Dewar-flask calorimeter.*

The averaged temperature of a sample immediately after exposure to radiation can be measured calorimetrically in the Dewar flask. Once the sample is added to the calorimeter, there is no change in the net energy of the calorimeter system. The energy change in the sample and in the sample container are exactly balanced by the energy change in the calorimeter. Using equation (1), this energy exchange relationship can be described by:

$$(M_s \times C_s + M_{sc} \times C_{sc}) \times (T_e - T_f) = (Z_d + Z_p + M_m \times C_m + M_1 \times C_1) \times (T_f - T_i) \qquad (2)$$

where the mass and specific heat of the sample are denoted by M_s and C_s; the mass and specific heat of the sample container by M_{sc} and C_{sc}; the mass and specific heat of the magnetic bar by M_m and C_m; and the mass and specific heat of the liquid medium used in the Dewar flask by M_1 and C_1; Z_d and Z_p are the heat capacities of the Dewar flask and of the temperature probe, respectively. The symbols, T_i, denote the temperature of the calorimeter's contents just before the sample is added, and T_f denotes the temperature just after equilibrium is reached; T_e represents the averaged temperature of the sample immediately after exposure to microwave radiation.

The parameters in equation (2) were obtained in the following manner. The temperatures of the calorimeter were measured directly, as were the masses of the various components. The heat capacity of the Dewar flasks, which were usually filled with 300 ml of water, was computed from the temperature changes observed when 50 ml of water at another temperature was added to the flasks. The specific heat of water is assumed to be 4.18 J/°C/g (1.0 cal/°C/g) between 20 °C and 30 °C. The specific heat or heat capacity of the other components was determined in a similar manner, using the water-filled Dewar flasks.

Corrections can be made in the values of T_i and T_f to eliminate the effect of thermal energy flowing into or out of the Dewar flask during the time when the flask is coming to equilibrium (see Figure 1). A simplified procedure [see *Hart*, 1951] -- if the calorimetric system is in a constant-temperature room -- is to determine the rate of temperature change for the calorimeter just before addition of the sample, r_1, and the rate of temperature change for the calorimeter just after equilibrium is reached, r_2. On the assumption that the temperature change in the calorimeter that is due to the addition of the specimen follows an approximation to an exponential time course, the time for the first 40% of the temperature change due to the sample addition is multiplied by r_1 (in appropriate units) and added to T_i. Similarly, the product of r_2 and the time taken for the last 60% of the temperature change is subtracted from T_f. A more detailed discussion of this technique and other correction treatments can be found in *Glazebrook* [1922] and *Dickinson* [1914].

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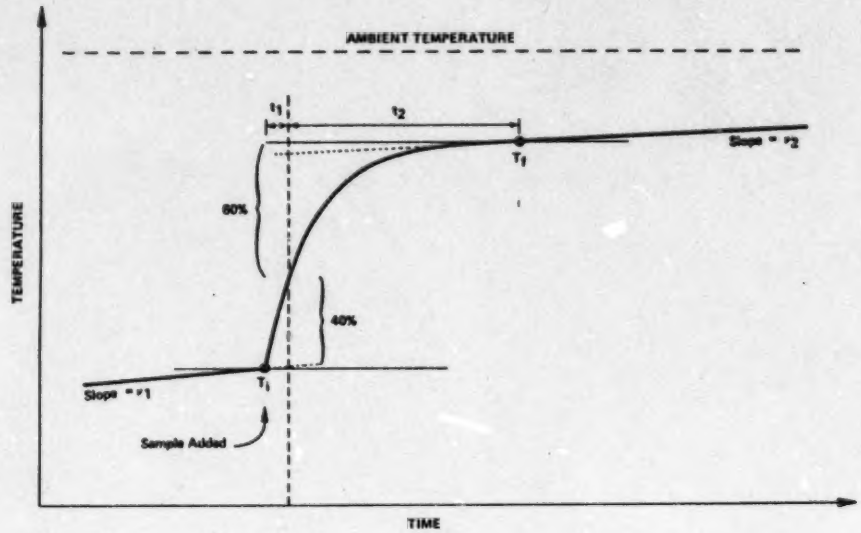

Fig. 1. Temperature in a calorimeter as a function of time. The solid bold line depicts a typical profile of temperature. Other lines are used as described in text to correct for heat exchange between the calorimeter and the environment.

3.2. *Electrical measurement of dose rate.*

The rate of energy absorption by a sample exposed in the transmission-line system is equal to the forward power minus the reflected and transmitted power plus the line losses. Two T-flasks filled with 40 ml of 0.15 M KCl solution at known temperatures were placed in the transmission line to provide a balanced load. A container of foamed polystyrene was not used in this apparatus because of limitations of space. Heat losses from the T-flasks during the two-minute exposures were found to be negligible because the exposure time was short and the temperature difference between the T-flask and the environment was small (less than 3 °C). The energy-absorption values determined by electrical measurements were normalized to the kilogram, with the assumption that the plastic flask absorbed no energy. Following exposure the T-flasks were placed in the Dewar-flask calorimeter and rates of absorption of energy were determined for comparison.

3.3. *Direct reading of sample temperatures.*

A T-flask fitted with a temperature probe [*Allis et al.*, 1977] was used as another means to obtain the initial and final temperatures of the sample. To reduce heat loss from the flask to the surroundings, the flask was placed in a foamed polystyrene container. A small bubble remained in the flask when it was filled with 40 ml of 0.15 M KCl solution. This bubble aided thermal mixing when the container enclosing the sample was rotated before and after the exposure. Only two to four minutes of rotation were needed in order to reach a stable temperature; T_i and T_f were read directly and were used to calculate a rate of energy absorption (as described above).

3.4. *Specific heat of Chinese hamsters.*

In order to use equations (1) and (2) to determine the energy absorbed by bodies of animals, the composite specific heat of the animal had to be determined. This was accomplished by the following procedure: the animal was killed in ether and enclosed in plastic wrap (Saran), which was sealed with a hot iron after air bubbles were removed by suction. Two animals so prepared were then put in a plastic bag with a temperature probe and were placed either in an ice bucket or in an incubator at 45 °C for periods of time to two hours. Upon reaching a steady-state temperature as recorded by the probe, the sealed animals were removed and were placed in appropriately prepared Dewar-flask calorimeters. The animals weighed between 30 and 40 g. Hence, 300 ml of water was used in the Dewar flasks to ensure that the animals were submerged as far as possible.

3.5. *Microwave energy absorbed by Chinese hamsters.*

The procedure to determine the averaged quantity of absorbed energy by the Chinese hamsters is the same as that described previously for T-flasks, except for the problem of internally generated heat from catabolic processes. This thermal contribution was minimized by cooling two animals of similar size in ice water enclosing both in similar insulated containers and exposing

only one to radiation. Following the two-minute exposure, both animals were removed from their containers and were placed in Dewar flasks. The averaged temperature of the unexposed animal was determined and compared to that of the exposed animal; equation (1) was used to determine absorbed energy. The procedure to minimize the effects of catabolic heat did cause an additional perturbation and is discussed later.

4. RESULTS

Typical values for the constants in the Dewar-flask calorimetric systems as used in these experiments are shown in Table 1. The heat capacity of the temperature probe is essentially zero; thus, it was omitted from the table.

A representative plot of the change in the calorimeter's temperature that was caused by adding a sample is shown in Figure 1. Temperature corrections can be made as described in Section 3 to adjust for heat exchange between the flask and the environment. Although this correction was not performed on any of the data presented here, the day-to-day change in ambient temperature contributed primarily to larger standard errors.

Figure 2 shows the rate of energy absorption for a T-flask exposed to various intensities of 2.45-GHz radiation for five minutes. Data from the Dewar-flask calorimeter were converted to rate of energy absorption (W/kg) and were plotted as a function of the measured power density (mW/cm^2). A regression analysis of these data gives a slope of 0.75 ± 0.04 *SE*(n = 12) and an intercept of 3.1 ± 2.7 *SE*(n = 12). This analysis shows that when the T-flask is exposed to 2.45-GHz radiation at a power density of 50 mW/cm^2, it absorbs 480 joules in five minutes at an averaged rate of 40 W/kg.

As a direct comparison, the T-flask fitted with an embedded probe was exposed under the same conditions. Direct measurements of the flask temperature before and after the exposure gave an equivalent energy-absorption rate of 40 ± 2 *SE* W/kg (n = 12), which also corresponds to an absorption of 480 joules of energy during the five-minute exposure.

A transmission-line exposure system, in which the rate of energy absorption could be measured electrically, was used as the final test of the accuracy of the Dewar-flask calorimeter. The T-flasks were exposed to 1.0-GHz

TABLE 1. Constants for calorimetric system.

Item	Mass (gm)	Sp. Heat (J/°C/gm)	Heat Capacity (J/°C)
Dewar Flask	---	---	171
Mag. Bar	3.9	0.67	2.59
T Flask	11.7	0.71	8.32
Chinese hamster	---	3.34	---
Water	300	4.18	1254
0.15 M KCl	40	~4.18	167

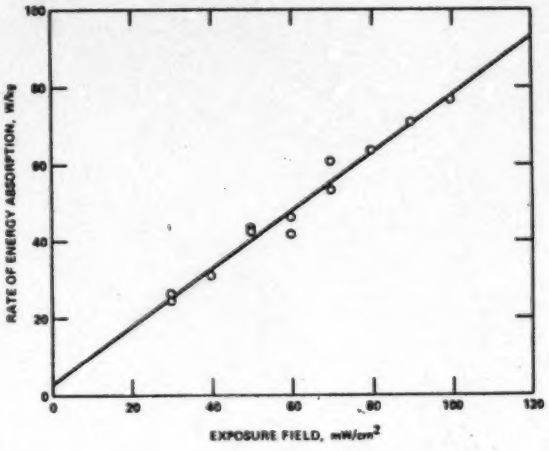

Fig. 2. Rate of energy absorption as a function of power density. Data are for a T-flask filled with 40 ml of 0.15 M KCl solution as exposed for five minutes to 2.45-GHz CW radiation.

radiation for two minutes at an indicated absorption rate of 100 W/kg. Analysis by the Dewar-flask calorimetric method gives an averaged rate of energy absorption of 101 ± 3 *SE* W/kg (n = 24). Some of the variation seen in these results was caused by fluctuations in the power level from the RF generator, rather than by errors associated with the calorimetric procedure.

Only a few experiments were conducted with dead-animal bodies to determine the feasibility of using our system for whole animal samples. The composite specific heat of 30- to 45-g Chinese hamsters, obtained from both heated and chilled bodies, was 3.34 ± 0.08 *SE* J/°C/g (n = 8) (0.80 ± 0.02 *SE* cal/°C/g (n = 8)).

The absorbed energy was obtained for 35- to 40-g Chinese hamsters exposed for five minutes to radiation at 100 mW/cm^2. For three animals that were exposed to 2.45-GHz plane waves, an incident power density of 100 mW/cm^2 gave an energy absorption rate of 38 ± 2 *SE* W/kg (n = 3).

5. DISCUSSION

Our study was undertaken to determine if a simple and inexpensive method can give reasonably accurate estimates of averaged quantities of absorbed energy for two types of samples after exposure to electromagnetic fields. Because the procedure uses temperature differences, it measures only the absorbed energy that is thermalized, which one presumes to approximate 100%. A limitation is that the quantity is a whole-body integral and does not address the question of differential deposition of energy within the specimen. As long as this limitation is recognized, the averaged value of absorbed energy can be used as a basis for comparing various experimental results.

There are other thermal procedures for measuring

absorbed energy. The most common of these are: direct measurement of the initial and final temperature of a biological specimen or of a phantom filled with a conductive solution; analysis of heating and cooling curves; and twin-well calorimetry. Each of these procedures has limitations that do not apply to the Dewar-flask calorimetric procedure.

The least expensive and most direct method for estimating absorbed energy is by direct measurement of temperatures of the sample. Collection and analysis of data can be done quickly (within 10 minutes), although only two data points are used for a determination. Because liquid samples are examined most easily with the least error, saline-filled phantoms are occasionally substituted for complex samples such as whole animals. There is a danger that values obtained with saline-filled phantoms may not be a true indication of the energy absorbed by the actual sample, because a unique sample geometry, orientation, conductivity or dielectric value can greatly alter scattering characteristics. Indeed, we found that the absorption rate by a 40-g KCl solution in a T-flask was approximately twice the absorption rate by a 40-g Chinese hamster when both were exposed in a free field of the same intensity.

The heating and cooling curve method can be applied to the same liquid samples that are used in the direct method of measurement. However, instead of only two data points, the heating and cooling curves supply as many data points as may be desired for analysis. Data collection requires periods to 90 minutes and the subsequent analysis can take from 15 minutes to four hours, depending upon the available data-processing equipment. Thus, methods based on heating and cooling curves provide a more reliable analysis but at the expense of time. Neither method is readily applicable to nonliquid samples.

Both the twin-well and the Dewar-flask calorimetric methods can be applied to nonliquid samples, such as whole animals. The twin-well method requires as long as 16 hours for data collection and 15 minutes for analysis, compared to 1 hour and 5 minutes, respectively, for the Dewar-flask method. Twin-well calorimeters must be custom-made at greater expense, while the Dewar-flask system usually can be assembled from equipment that is available in most biochemical or physical-chemical laboratories. The sensitivity of the twin-well method is theoretically greater than the Dewar-flask method; however, in practice the components of the Dewar-flask calorimetric system can be readily and inexpensively changed to maintain a higher average sensitivity for whatever sample size and exposure range is being utilized. Such component changes include reducing the amount of heat exchange medium in the vacuum flask, reducing the size of the flask, or replacing the usual aqueous heat-exchange medium with a liquid of lower specific heat and density. One possible substitute for water is dodecane, which has a specific heat of 2.1 J/°C/g (0.5 cal/°C/g) and a density of 0.75 g/cm^3. Although this substitution would increase any temperature change by a factor of 2.7, dodecane needs to be treated with caution because of its low flash point of 74 °C.

The ability of the Dewar-flask method to measure accurately the averaged rate of energy absorption for an *in vitro* sample was amply demonstrated with T-flasks. At a power density of 50 mW/cm^2, the Dewar-flask method and the direct method gave an averaged rate of energy absorption of 40 W/kg. In the transmission-line exposure system where the electrically determined rate of energy absorption was 100 W/kg, the Dewar-flask method measured 101 W/kg. Finally, for the heating/cooling curve analysis described by *Allis et al.* [1977], a series of power-density measurements gave a regression line with a slope of 0.74 ± 0.02 *SE* (n = 48) and an intercept of 0.13 ± 0.67 (n = 48). This can be compared with a regression slope of 0.75 ± 0.04 *SE* (n = 12) and an intercept of 3.1 ± 2.7 *SE* (n = 12) found independently with the Dewar-flask method. The intercept value of 3.1, although not statistically different from zero, reflects a slight contribution from the higher temperature of the environment. Corrections for this perturbation should be applied as described in Figure 1 and in the text, if extrapolations to lower power densities are attempted.

The ability of the Dewar-flask method to measure accurately composite specific heat and averaged absorbed energy for whole animals has been examined. Good agreement is apparent for the measurement of composite specific heat of dead Chinese hamster bodies, 3.34 ± 0.08 *SE* J/°C/g (n = 8) (0.80 ± 0.02 *SE* cal/°C/g (n = 8)), when compared to the value reported for white mice, 3.444 ± 0.021 *SE* J/°C/g (n = 8) (0.824 ± 0.005 *SE* cal/°C/g (n = 15)) [*Hart*, 1951]. Only three hamsters were exposed to electromagnetic radiation, but the relation between power density and averaged rate of energy absorption, 10 mW/cm^2 = 3.8 ± 0.2 *SE* W/kg (n = 3) for 35- to 40-g animals, can be compared with 4.6 W/kg reported by *Huang et al.* [1977], who used twin-well calorimetry. Part of the difference between these two absorption rates may be due to exposure configuration and to temperature. The larger value cited by *Huang et al.* [1977] was a mean for five animals exposed simultaneously, a situation that usually gives a slightly larger mean absorption rate because of reflections between animals.

The effect of an animal's body temperature on the rate of energy absorption was examined. The bodies of white mice (approximately 25 g each) were exposed at 0 and at 38 °C in our transmission-line system at 1 GHz. The absorption rate at 38 °C of 1.80 ± 0.03 *SE* W/kg (n = 8) was found to be nearly 40% greater than the value of 1.29 ± 0.02 *SE* W/kg (n = 8) found at 0 °C. The most likely cause of this increase is the change in conductivity of body fluids between these two temperatures. If the temperature dependence of the absorption value for white mice is equivalent to that for Chinese hamsters, the absorption value for hamsters at 38 °C is 5.1 W/kg, based on our value of 3.8 W/kg at approximately 5 °C (the composite temperature of the unexposed animals rose from 0 to 10 °C during treatment). The dosimetry in Huang's study was performed on animal bodies at approximately 25 °C [*J.B. Kim*, 1977, personal communication]. Therefore, good agreement is found between the absorption rates deter-

mined by the Dewar-flask method and the twin-well method when the values are corrected for temperature. These results demonstrate the importance of specimen temperature when measuring rates of energy absorption.

Although the power densities used for the animal exposures were high (100 mW/cm^2), there are several ways to extend the sensitivity of the Dewar-flask method when using radiation at lower field strengths. In addition to reducing the volume of the heat exchange medium and to changing to dodecane, several similarly exposed animals may be placed in the flask simultaneously to increase the quantity of thermal energy being measured. In addition, the efficiency of thermal insulation of the container can be increased to further inhibit heat exchange and the animal can be exposed for longer periods of time. The Dewar-flask method is applicable to a variety of specimens and exposure conditions but requires selective changes in flask size, volume of liquid, sample containment and so forth in order to maximize the sensitivity for a given application.

As demonstrated, the Dewar-flask method is simple, inexpensive, accurate and widely applicable for determining averaged rates of energy absorption in biological studies of electromagnetic radiation. Its application is limited by the inability of the user to measure small increments of temperature and by power input, which must be fairly high. The method does not provide information on the distribution of energy deposition within a sample and may distort results to the extent that dead animals do not simulate live ones. In spite of these limitations, the advantages of Dewar-flask calorimetry are so numerous that the method could be considered a viable accompaniment to measurements of power density in many laboratories.

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13. TYPE OF MATERIAL:
a. ☐ Journal Publication
b. ☐ Oral Presentation
c. ☒ Paper for Conference Proceedings or Meeting
d. ☐ Other: _____

14. NAME & LOCATION OF JOURNAL, CONFERENCE, MEETING, ETC.:
Special Supplement to Radio Science, URSI Annual Meeting, Amherst, Mass, 11-15 October 1976

15. PRINCIPAL ORD AUTHOR: a. Signature

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IV. REVIEWS (Attach All Comments)

16. a. Technical Review ☒ Yes ☐ No ☐ N/A
b. Editorial Review ☒ Yes ☐ No ☐ N/A
c. Format Review ☐ Yes ☐ No ☐ N/A
d. Policy Review ☐ Yes ☐ No ☐ N/A

Reviewer(s) Name & Address:
1) Claude Weil, EBD, HERL
2) Ralph Smialowicz, EBD, HERL
3) Joseph Ali, EBD, HERL

e. TECHNICAL INFORMATION COORDINATOR: f. Signature M. Morrison

g. Typed Name & Address: M. Morrison, TIC, HERL | h. Date: 10/8/76 | i. Telephone: 2462

V. APPROVALS

LABORATORY	HEADQUARTERS
17. LINE SUPERVISOR	19. LINE SUPERVISOR
a. Signature D. Cahill b. Date 10/7/76	a. Signature b. Date
18. LABORATORY DIRECTOR J. H. Knelson, Director, HERL	20. DEPUTY ASSISTANT ADMINISTRATOR
a. Signature b. Date 10/11/76	a. Signature b. Date

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